Synthesis and Study of Antibacterial Activity of some New Bis-pyrazolines Derivatives

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Abstract

A series of novel bis-pyrazolines (13-24) were prepared by the reaction of two moles of substituted chalcones, diarylidene acetone and dienylidene acetone (1-12) in a Claisen 1, 2-addition with succinohydrazide. Some of the new compounds were characterized using ^{1}H, ^{13}C-NMR and all new compounds were characterized using FT- IR. The antibacterial activity of these compounds was studied.

Keywords: Claisen 1,2-addition, Bis-pyrazolines, Succinohydrazide, antibacterial activity.

الخلاصة

تم تحضير سلسلة من مركبات ثنائية حلقة االبيرازولين (13−24) من خلال تفاعل مولين من الجالكونات وأريليدينات الأسيتون الثنائية وأينياليينات الأسيتون الثنائية (1−12) بواسطة ميكانيكية أضافة كليزن 1−2 مع السكسنيك هيدرازايد وشخصت بعض المركبات الجديدة باستخدام طيف (^{1}H، ^{13}C−NMR) وشخصت جميع المركبات الجديدة باستخدام الأشعة تحت الحمراء.كما و تم تقييم النشاط المضاد للبكتيريا لهذه المركبات.

الكلمات المفتاحية: أضافة كليزن 1−2 , البايرازوليينات الثنائية, السكسنيك هيدرازايد, الفعالية المضاده للبكتريا.

Introduction

Five-membered heterocyclic compounds Pyrazolines are well known. Many researchers worked hard to develop different methods of synthesis to this compound. [Wiley, 1967., Elguero, 1996., Lévai, 1997.,Lévai,2002]. Many pyrazoline derivatives were found to possess considerable biological activities. Which have stimulated research activity in this field [Ramalingham, 1977., Brown, 1972., Lombardino, 1981., United States Patent, 2005., United States Patent, 1993., Conti, 2007., Basagoiti, 2006]. The reason caused by the two nitrogen atoms in the molecule. There are several methods to prepare Pyrazolines, the standard method includs the cyclocondensation of alkyl hydrazine or aryl hydrazine with α, β- unsaturated carbonyl compounds [Eichorand, 2005]

Experimental

All chemicals were purchased from Fluke and BDH Chemical Ltd. General: uncorrected melting points were determined using Electrothermal melting point apparatus (Electrothermal Engineering LTD S-N 10853). Infrared spectra were recorded on (Shimadzu FT-IR 8400 S, Fourier Transform-Infrared Spectrophotometer). Proton Nuclear Magnetic Resonance (^{1}H-NMR) and Carbon Nuclear Magnetic Resonance (^{13}C-NMR) spectra were recorded with Brucker (300 MHz) using tetramethylsilane (TMS) as an internal standard, and DMSO as a solvent.

Synthesis of diethyl Succinate.

Succinic acid (1.6 g) in absolute dry ethanol (60 ml), containing 2-3 drops of concentrated H_2SO_4 was relaxes until it dissolved. The reaction mixture was poured in ice-cold water. The last reaction mixture, sodium bicarbonate was added till the effervescence stopped .The obtained ester was extracted with diethyl ether. **[**Avaji, 2009]

Synthesis of dihydrazide of Succinic acid.

A mixture of diethyl ester of succinic acid (2.22 g) and hydrazine hydrate (80%) in ethanol was refluxed for 4-5 hs. The reaction mixture was allowed to cool at room temperature, then the cooled solution was poured into ice cold water. The dihydrazide of scenic acid, thus obtained was filtered and recrystallized from ethanol. **[**Robert, 2005]

Synthesis of Chalcones (1- 5)

Chalcones were prepared by a base catalyzed condensation of a mixture of acetophenones and substituted benzaldehydes in alcohol, 60% solution of potassium hydroxide (KOH) was added dropwise with stirring. The reaction mixture was kept at room temperature for 14-16 hs, then diluted with water and acidified with 10% hydrochloric acid. The chalcones that obtained was filtered and recrystallized from ethanol. **[**Fabio, 1998] Melting point, yield and color of these compounds are shown in Table (1).

Synthesis of Diaryledene and dienylidene acetone [Vogel, 1981]

*Typical procedure for the synthesis of compounds (**6-12**):*

A solution of acetone (1.4 g, 25 mmol) and benzaldehyde [(50 mmol) or substituted benzaldehyde and cinnamaldehyde or substituted cinnamaldehyde] was added dropwise with stirring at room temperature to a stirred solution of sodium hydroxide (5 g, mmol), in [water (50 mL) and ethanol (40 mL)]. The stirring was continued for 30 min at room temperature. The resulting precipitate was filtereds, with cold water washes (3x30 mL) and dried. Recrystallization from ethanol (95%) afforded the required product. Melting point and color of these compounds are shown in Table (1).

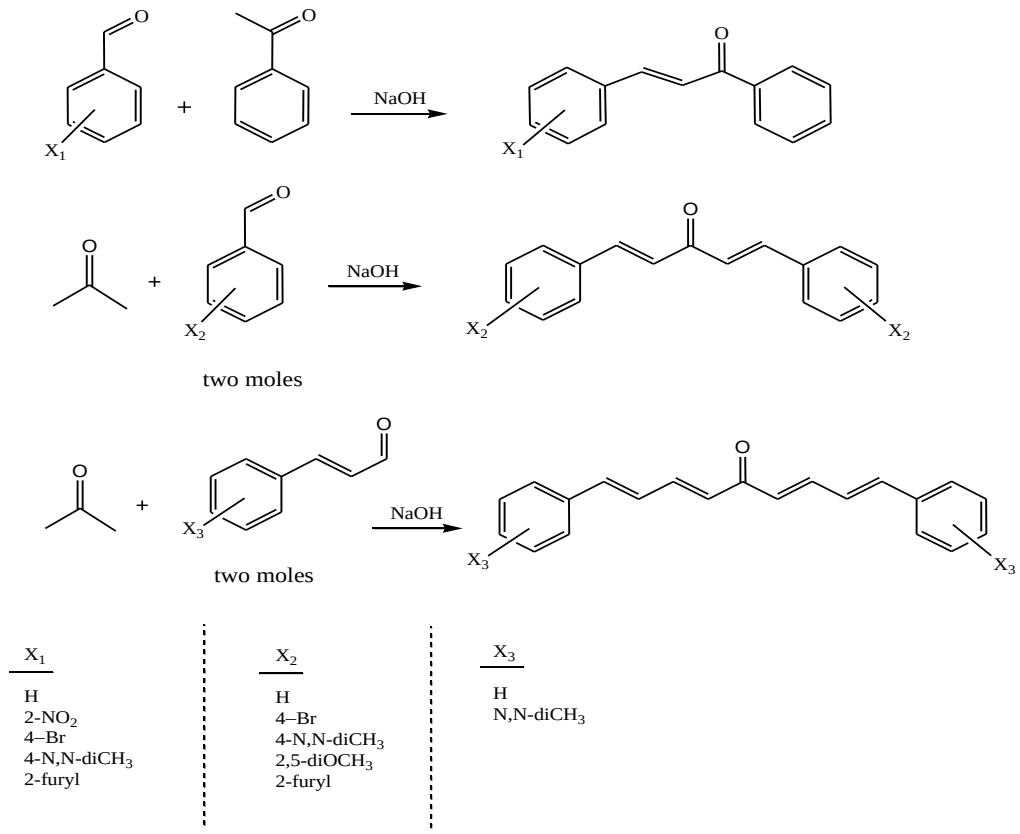
Synthesis of substituted bis-pyrazolines (13-24)

A mixture of chalcone, (diaryledene and dienylidene) acetone (1–12, 10.0 mmoles), succinohydrazide (50.0 mmoles), and acetic acid (60 ml) heated at reflux for 3hs, then poured onto crushed ice. The precipitate was separated by filtration, with water washes and crystallized from methanol to obtain the 2-pyrazolines **[**Albert Lévai (2005)]. Melting point, yield and color of these compounds are shown in Table (2).

Results And Discussion

2.1- Condensation of aldehydes with ketones

Benzaldehydes, substituted benzaldehydes, cinnamaldehyde and substituted cinnamaldehyde were condensed with ketones containing α- hydrogen by Claisen-Shmdit condensation in the presence of NaOH to afford Chalcones, Diaryledene acetone and dienylidene acetone respectively. Through the reaction access to the desired products (1-12) (Scheme 1). These compounds were fully characterized using FT-IR (Table 4), ^{1}H-NMR, and ^{13}C-NMR.



Scheme 1: Synthesis of substituted α, β- unsaturated ketones

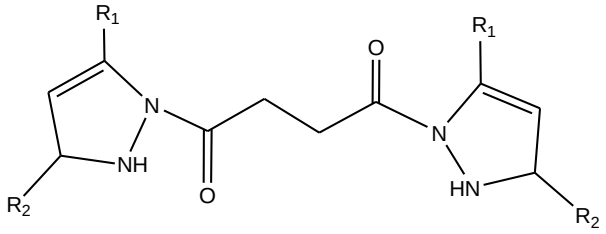
FT-IR spectra of the compounds (1-12) showed a sturdy uptake band in the (1648-1681 cm -1), denote to extend bumping of the carbonyl group (νC = O). Conjugation of carbonyl group with (C=C) led to in a lack of concentration for a carbonyl group, which led to the uptake of the transition to a lower hesitancy. [Silverstein, 1996]. packs at (1610-1579 cm-1) denote to (C=C) bumping extend (νC=C) and the packs at (1590-1477 cm-1) denote to aromatic ring extend bumping (νC=C).

The (^{1}H-NMR) spectrum of compound (7) showed a peak at δ (7.03) ppm (2H) denote to the olefinic protons at C_2 and C_4, the multiple peak at δ (7.28-7.63) ppm (8H) denote to the aromatic protons, while the peak at δ (7.70) ppm (2H) denote to the olefinic protons at C_1 and C_5. The (^{1}H-NMR) spectrum of compound (9) showed a peak at δ (3.85) ppm (12H) Denote to the methoxy protons, the peak at δ (6.29) ppm (2H) denote to the aromatic protons at C_4 while the peak at δ (7.16) ppm (4H) Denote to the aromatic protons at C_3 and C_5, another peek at δ (7.98) ppm (2H) denote to the olefinic protons at C_2 and C_4, finally the peak at δ (8.03) ppm (4H) denote to the olefinic protons at C_1 and C_5. The ^{13}C-NMR spectrum of compound (9**)** showed a peak at δ (55.82) ppm denote to the methoxy carbon at the ortho position in the two rings; another peak at δ (56.10) ppm denote to the methoxy carbon at the meta-position in the two rings. There are four peaks at δ (112.43) ppm, δ (113.17) ppm, δ (117.19) ppm, and δ (124.46) ppm, denote to the *o, p, m* carbon atoms attached to the two olefinic carbon at C_2 and C_4. There is a peak at (138.07) ppm denote to the ortho carbon attached to the methoxy group of the two rings. The peak at δ(153.12) ppm denote to the olefinic carbon at C_1 and C_5, while the peak at δ(153.50) ppm denote to the meta carbon attached to the methoxy group of the two rings; finally the peak at

δ(189.58) ppm denote to the carbonyl carbon.^{13}C-NMR data of compound (6**)** is summarized in (Table 5).

2.2- Condensation of Chalcones, Diaryledene acetone and dienylidene acetone with dihydrazide of Succinicacid:

The condensation of Chalcones, Diaryledene acetone and dienylidene acetone with dihydrazide of scenic acid afforded substituted -2-pyrazolines (13-24).

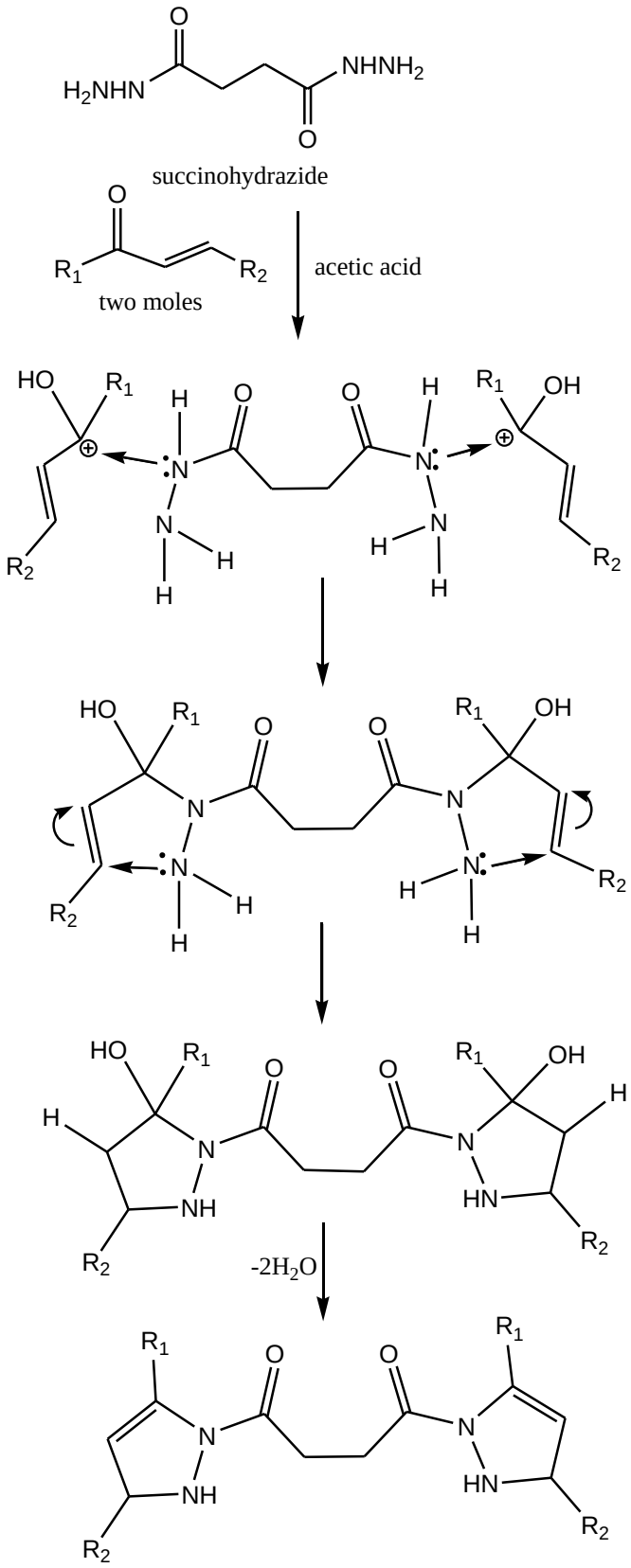


The structures of the products were confirmed on the principle of spectroscopic evidences (table 4). The (FT-IR) spectra was shown strong or medium bands at (1710-1692) cm^{-1} corresponding to the (υC=O), which mean removal of the conjugation of carbonyl group with (C=C) [Silverstein, 1996]. The bands at (1519 -1486) were corresponding to the extend bumping of (υC=C) bond, while the bands at (3430-3389) cm^{-1} corresponding to the extend bumping of (υN-H) bond and the bands at (1542-1485) denote to the aromatic ring extend bumping of (υC=C).

While the (^{1}H-NMR) spectrum of compound (15) (figure 3) show a peak at δ(2.52) ppm (1H) denote to the proton of N-H group**[**Rahaman, 2010]. Another peak at δ(3.37) ppm (4H) denote to the methylene groups at C_2 and C_4 from the dihydrazide of Succinic acid part. The triplet peak at δ(4.80, 4.85, 4.89) ppm (1H) denote to the methine groups at C_3 of two pyrazoline rings . Finally the multiplet peak at δ(6.90-7.85) ppm (20H) denote to the olefinic protons at C_4 of pyrazoline rings and aromatic protons.

The ^{13}C-NMR spectrum of compound (15**)** shown a peak at δ (35.72) ppm denote to the C_2 and C_4 of methylene carbon from the dihydrazide of succinic acid part. Another peak at δ (65.19) ppm denote to the C_3 of two pyrazoline rings. The peak at δ (103.64) ppm denote to the C_4 of two pyrazoline rings. There are eight peaks at δ (123.56) ppm, δ (128.78) ppm, δ (131.25) ppm, δ (132.57) ppm, δ (133.58) ppm, δ (134.25) ppm, (136.79) ppm and δ (145.65) ppm denote to the carbon atoms of benzene rings. There is a peak at (139.32) ppm denote to C_4 of two pyrazoline rings. Finally the peak at δ(175.36) ppm denote to to the carbonyl carbon.

The suggested mechanism for the reaction of Chalcones, Diaryledene acetone and dienylidene acetone with succinohydrazide may follow by attacking the nitrogen atom of succinohydrazide of the α, β-unsaturated system (scheme 2).



R_1	**R_2**
Ph	Ph
2-$NO_2C_6H_4$	Ph
4-BrC_6H_4	Ph
4-N,N-$CH_3C_6H_4$	Ph
2-furyl	Ph
Ph	$C_6H_5CH=CH$
4-BrC_6H_4	4-$BrC_6H_5CH=CH$
4-N,N-$CH_3C_6H_4$	4-N,N-$CH_3C_6H_5CH=CH$
2,5-di$OCH_3C_6H_3$	2,5-di$OCH_3C_6H_4CH=CH$
2-furyl	2-vinyl-2-furyl
$C_6H_5CH=CH$	$C_6H_5CH=CH-CH=CH$
4-N,N-di$CH_3C_6H_5CH=CH$	4-N,N-di$CH_3C_6H_5CH=CH-CH=CH$

Scheme (2): Reaction of substituted α,β- unsaturated ketones with succinohydrazide

Preliminary biological study

In the present study biological effect of the chemical products were performed against four gram-negative bacteria: *Escherichia coli*, *Pseudomonas aeruginosa*, *Citrobacter freundii*, *Morganella morganii* and two gram-positive bacteria: *Staphylococcus aureus*, *Staphylococcus saprophyticus* and one species of yeast *Rhodotorula harrison* (Table 6).

All these micro-organisms were isolated and identified in Medical Laboratory Techniques Department/ Technical College in Kirkuk. Bacterial suspension was prepared by transferring a single colony to a fresh test tube contained 5ml nutrient broth the incubated at 37ºC for 24 hours. Bacterial suspension compare with the tube NO. (0.5) of McFarland standard was used which gives a cell density 1.5×10^8 cell/ml. (Baron *et al.*, 1994).

A sterile cotton swab is dipped into bacterial suspension and then swabbed evenly across the surface of a Mueller-Hinton agar plate; the plates were incubated at (37ºC) for 30 minutes. Saturated disks (made from Whatman NO. 1 impregnated for 24 hours with the chemical products 0.1mg / 1ml) are applied on Mueller-Hinton agar using Kirby–Bauer disc diffusion method **[**Bauer, 1966] with a forceps pressed firmly to ensure contact with agar and then the plates inverted and incubated at (37ºC) for (14-18) hours, reading the results by measuring inhibition zone around the discs and compare the result with two antibiotics used as control [Ericsson, 1960]. The results were interpreted to rely on the World Health Organization report (W.H.O).

[Vandepitte, 1991].

I-The impedance (I) result represented the diameter of inhibition (≤15 and ≤14) mm for Ciprofloxacin and Cefotaxime respectively.

II- Moderate sensitivity (MS) inhibition within the area between (16-20 and 15-22) mm.

III- The sensitive (S) result was (≥21 and ≥23) mm.

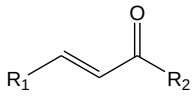


Table (1): Physical properties of the synthesized compounds (1-12)

Cpd. No.	R_1	R_2	M.P ˚C	Yield %	Color	Product Name
1	Ph	Ph	52-54	67	yellow	1,3-Diphenyl-2-propen-1-one
2	2-$NO_2C_6H_4$-	Ph	149-151	63	dark blue	3-(2-nitrophenyl)-1-phenylprop-2-en-1-one
3	4-Br C_6H_4-	Ph	83-85	80	white	3-(4-bromophenyl)-1-phenylprop-2-en-1-one
4	4-N,N-CH_3 C_6H_4-	Ph	89-91	75	yellow	3-(4-(dimethylamino)phenyl)-1-phenylprop-2-en-1-one
5	2-furyl	Ph	37-39	82	brown	3-(furan-2-yl)-1-phenylprop-2-en-1-one
6	Ph	C_6H_5CH=CH-	107-109	80	yellow	1,5-diphenylpenta-1,4-dien-3-one
7	4-Br C_6H_4-	4-BrC_6H_5CH=CH-	165-166	90	white	1,5-bis(4-bromophenyl)penta-1,4-dien-3-one
8	4-N,N-CH_3 C_6H_4-	4-N,N-CH_3 C_6H_5CH=CH-	114-116	85	orange	1,5-bis(4-(dimethylamino)phenyl)penta-1,4-dien-3-one
9	2,5-DiOCH_3 C_6H_3-	2,5-DiOCH_3 C_6H_4CH=CH-	98	82	yellow	1,5-bis(2,5-dimethoxyphenyl)penta-1,4-dien-3-one
10	2-furyl	2-vinyl-2-furyl	150-152	42	brown	1,5-di(furan-2-yl)penta-1,4-dien-3-one
11	C_6H_5CH=CH-	C_6H_5CH=CH-CH=CH-	130-132	80	yellow	1,9-diphenylnona-1,3,6,8-tetraen-5-one
12	4-N,N-CH_3 C_6H_5CH=CH-	4-N,N-CH_3 C_6H_5CH=CH-CH=CH-	123-125	79	brown	1,9-bis(4-(dimethylamino)phenyl)nona-1,3,6,8-tetraen-5-one

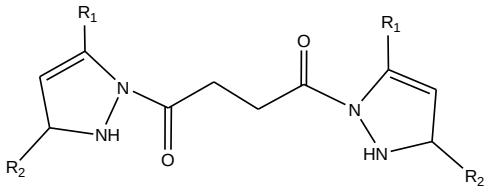


Table (2): Physical properties of the synthesized compounds (13-24)

Cpd. No.	R_1	R_2	M.P ˚C	Yield %	Color	Product Name
13	Ph	Ph	110-113	80	green	1,4-bis(3,5-diphenylpyrazolin-1-yl)butane-1,4-dione
14	2-$NO_2C_6H_4$-	Ph	178-180	85	Pale green	1,4-bis(3-(2-nitrophenyl)-5-phenylpyrazolin-1-y)butane-1,4-dione
15	4-Br C_6H_4-	Ph	124-125	72	Pale yellow	1,4-bis(3-(4-bromophenyl)-5-phenylpyrazolin-1-y)butane-1,4-dione
16	4-N,N-diCH_3 C_6H_4-	Ph	283-286	65	brown	1,4-bis(3-(4-N,N-dimethylphenyl)-5-phenylpyrazolin-1-y)butane-1,4-dione
17	2-furyl	Ph	162-164	82	Dark brown	1,4-bis(3-(furan-2-yl)-5-phenylpyrazolin-1-y)butane-1,4-dione
18	Ph	C_6H_5CH=CH-	119-122	90	Dark yellow	1,4-bis(phenyl-5-styrylpyrazolin-1-y)butane-1,4-dione
19	4-Br C_6H_4-	4-BrC_6H_5CH=CH-	117-118	69	Pale yellow	1,4-bis(3-(4-bromophenyl)-5-(4-bromostyryl)pyrazolin-1-y)butane-1,4-dione
20	4-N,N-diCH_3 C_6H_4-	4-N,N-CH_3 C_6H_5CH=CH-	66-69	38	Dark red	1,4-bis(3-(4-N,N-dimethyaminolphenyl)-5-(4-N,N-dimethylaminostyryl)pyrazolin-1-y)butane-1,4-dione
21	2,5-diOCH_3 C_6H_3-	2,5-diOCH_3 C_6H_4CH=CH-	135-137	45	Yellowsh green	1,4-bis(3-(2,5-dimethoxy phenyl)-5-(2,5-dimethoxystyryl)pyrazolin-1-y)butane-1,4-dione
22	2-furyl	2-vinyl-2-furyl	126-128	80	Pale brown	1,4-bis[3-(furan-2-yl)5-(2-(furan-2-yl)vinyl)pyrazolin-1-yl]buta1,4-dione
23	C_6H_5CH=CH-	C_6H_5CH=CH-CH=CH-	102-104	90	Faint green	1,4-bis(5-(-4-phenylbuta-1,3-dienyl)-3-styrylpyrazolin-1-y)butane-1,4-dione
24	4-N,N-CH_3 C_6H_5CH=CH-	4-N,N-CH_3 C_6H_5CH=CH-CH=CH-	96-99	77	mauve	1,4-bis(3-(4-(dimethylamino)styryl)-5-(4-(4-(dimethylamino)phenyl)buta-1,3-dienyl)pyrazolin-1-y)butane-1,4-dione

Table (3): FT- IR data of compounds (1-12).

Cpd. No.	I.R (KBr), υ(cm^{-1})			
	C=O	C=C	Ar.C=C	Others
1	1675	1593	1500	
2	1655	1590	1487	N=O/sym.,asy.=1339,1518
3	1663	1587	1481	------
4	1655	1589	1477	------
5	1651	1579	1485	------
6	1681	1602	1492	------
7	1650	1610	1488	------
8	1660	1610	1580	------
9	1650	1589	1492	C-O-C = 1218
10	1648	1598	1518	------
11	1655	1600	1525	------
12	1659	1605	1590	------

Table (4): FT- IR data of compounds (13-24).

Com. NO.	I.R (KBr), υ(cm^{-1})				
	C=O	C=C	N-H	Ar.C=C	Others
13	1699	1517	3415	1509	------
14	1704	1514	3430	1492	N=O/sym.,asy.=1390,1520
15	1697	1519	3390	1500	------
16	1700	1510	3408	1542	------
17	1709	1518	3395	1495	------
18	1692	1507	3402	1499	------
19	1695	1515	3409	1508	------
20	1710	1499	3389	1540	------
21	1697	1509	3412	1492	C-O-C = 1228
22	1703	1512	3416	1490	------
23	1705	1510	3420	1505	------
24	1697	1486	3428	1485	------

Table (5): ^{13}C-NMR data of compounds (6, 9, 15).

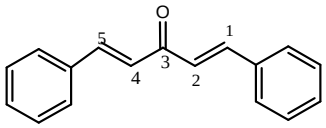
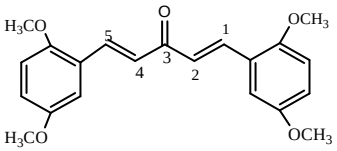
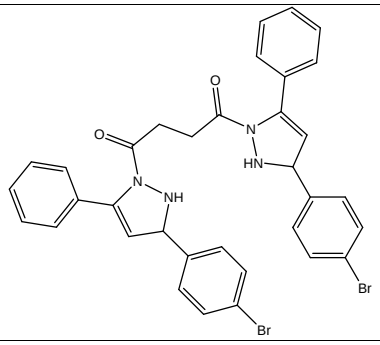
Cpd. No.	Structures	^{13}C-NMR ($CDCl_3$ & DMSO), δ ppm						
6		125.67 C_2 & C_4	128.28 four ortho-positions	128.25 two para-positions	130.49 four meta positions	134.26 two carbons attached with α,β-sys.	142.74 C_1 & C_5	188.50 C_3
9		55.82 ortho-OCH_3	56.10 meta OCH_3	112.43 un substituted ortho-positions	113.17 two para-positions	117.19 un substituted meta-positions	124.46 two carbons attached with α,β-sys.	126.30 C_2 & C_4
		138.07 substituted ortho-positions	153.12 C_1 & C_5	153.50 substituted meta-positions	189.58 C_3	-----	-----	-----
15		35.72 C_2 & C_4 of CH_2 of hydrazide	65.19 C_3 of pyrazoline rings	103.64 $C_4$of pyrazoline rings	123.56 128.78 131.25 132.57 133.58 134.25 136.79 145.65 C of benzene rings	139.32 $C_4$of pyrazoline rings	175.36 C of carbonyl	-----

Table (6): Inhibition effect of products on the growth of some bacteria and yeast.

Cpd. No.	Test bacteria						yeast
	Gram +ve		Gram -ve				
	Staphylococcus aureus	*Staphylococcus saprophyticus*	*E. coli*	*Pseudomonas aeruginosa*	*Citrobacter freundii*	*Morganella morganii*	*Rhodotorula harrison*
13	MS	MS	I	I	I	I	I
14	MS	I	I	I	I	I	I
15	I	I	I	I	I	I	I
16	I	I	I	I	I	I	I
17	I	I	I	I	S	I	S
18	S	S	I	I	S	I	S
19	MS	MS	I	I	I	I	S
20	MS	MS	I	I	I	I	I
21	I	I	I	I	I	I	I
22	I	I	I	I	I	S	I
23	I	I	I	I	I	S	I
24	S	S	I	I	I	S	I
Ciprofloxacin (5ug)	MS	MS	S	S	S	S	---
Cefotaxime (30ug)	I	I	I	I	I	I	---

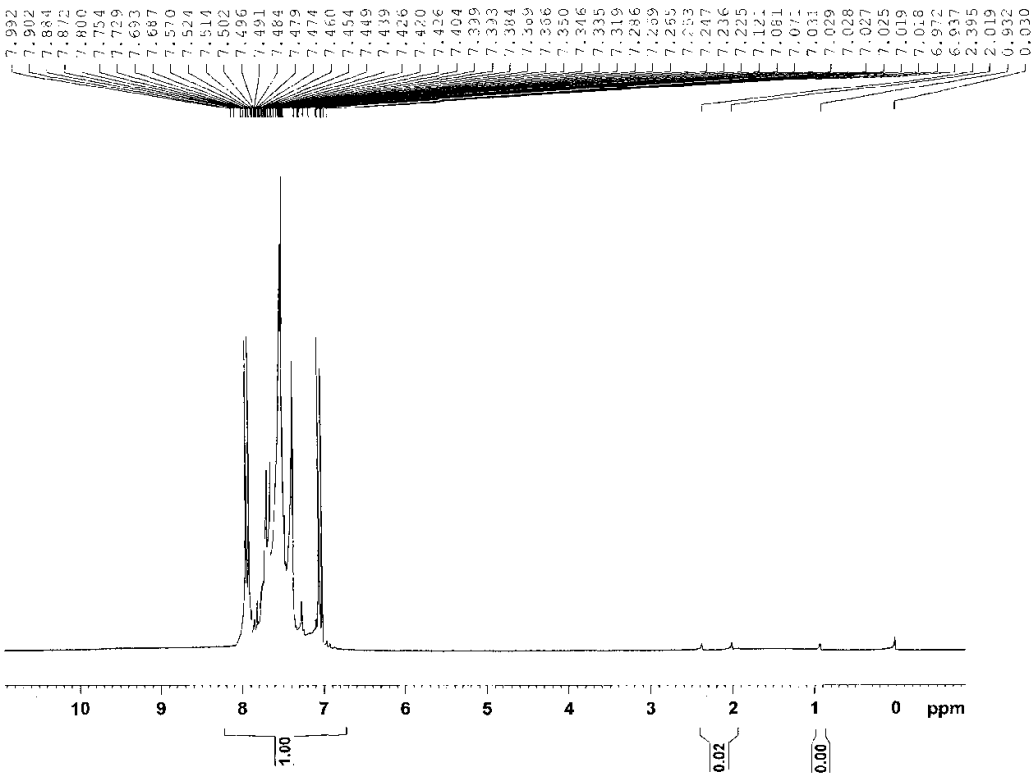


Fig. (1): ^{1}H-NMR spectrum of compound (7).

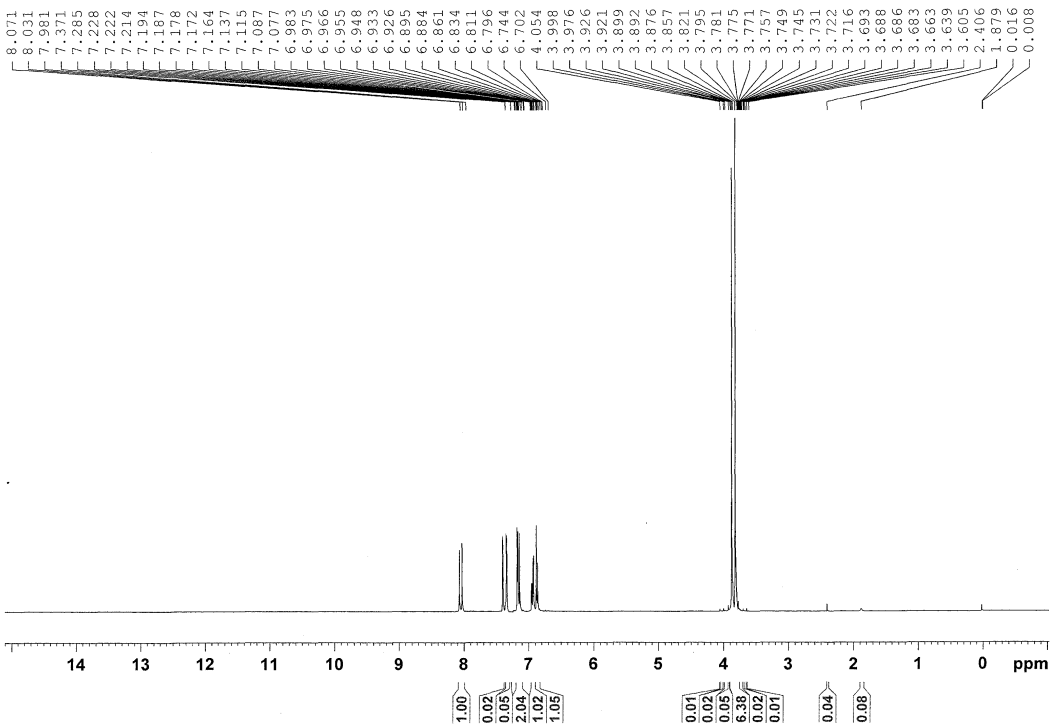


Fig. (2): ^{1}H-NMR spectrum of compound (9).

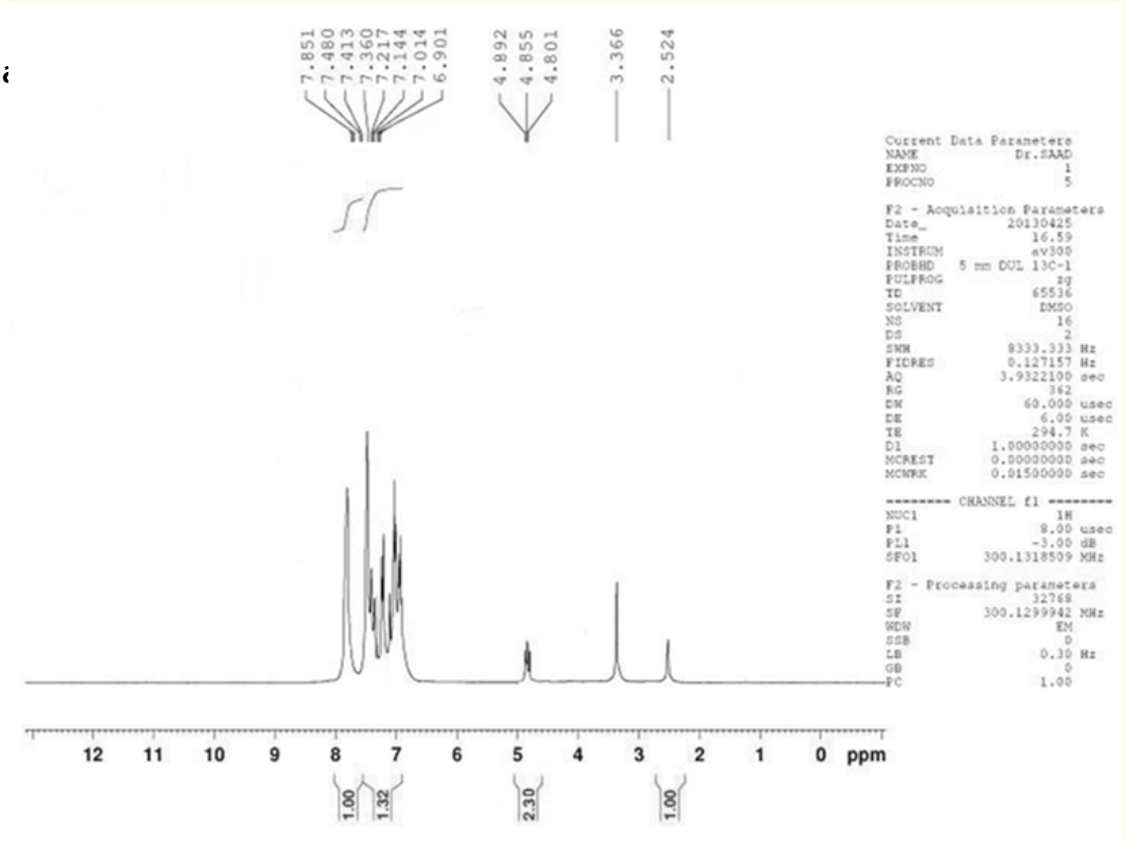


Fig. (3): ^{1}H-NMR spectrum of compound (15).

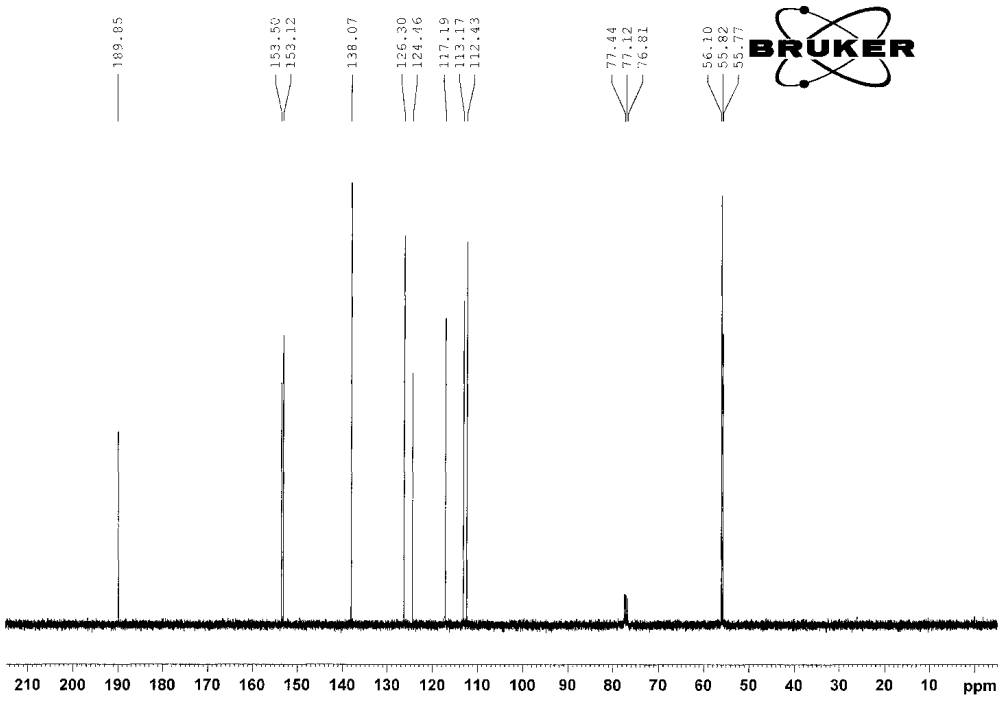


Fig. (4): ^{13}C-NMR spectrum of compound (9).

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